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Characterisation of the Omsk collection of rickettsial strains

N. V. Rudakov[1], S. N. Schpynov[1], I. E. Samoylenko[1], P.-E. Fournier[2], T. A. Reschetnikova[1], L. V. Kumpan[1] and D. Raoult[2]

[1]Omsk Research Institute of Natural Foci Infections, Omsk State Medical Academy, prospect Mira, Omsk, Russia and [2]URMITE, CNRS-IRD UMR, Universite de la Mediterranee, Faculte de Medecine, Bd Jean Moulin, Marseille, Cedex, France

INTRODUCTION

North Asian tick typhus (NATT) was the first tick-borne rickettsiosis detected in Russia. To date, more than 65 000 NATT cases have been registered. Since 1954, the Omsk Institute of Natural Foci Infections has cultivated rickettsiae from ticks and clinical specimens collected in natural foci of NATT as part of the study of the epidemiology of this disease and the ecology of tick-borne rickettsiae [1]. Herein, we used gene amplification and sequencing to identify 50 strains with accuracy and thereby describe the rickettsial species endemic in NATT foci in Russia.

MATERIALS AND METHODS

Ticks collected in NATT foci were inoculated into guinea pigs or chicken embryos. Rickettsial strains were subsequently maintained in Vero cell lines or by means of modeling natural cycles of metamorphosis of their tick vectors [2].

Rickettsial strains prevalent in NATT foci were identified using gene sequencing. DNA was extracted using the QiaAmp Tissue kit as recommended by the manufacturer (Qiagen, Hilden, Germany). PCR amplification was performed using the 190-70 and 190-701 primer pair for the *ompA* gene (590 bp), and both the CS1d - CS535r and CS409d - RP1258n pairs for the *gltA* gene (1234 bp). Sequencing of positive PCR products was performed using the D-rhodamine terminator cycle DNA sequencing kit and an ABI Prism 3100 automated Sequencer (Applied Biosystems, Foster City, CA, USA) [3,4]. All sequences were performed twice in both directions.

RESULTS

Twenty-seven isolates were identified as *R. sibirica* subsp. *sibirica* (24 isolated primarily in guinea pigs, one in a hamster and two in chicken embryos), three as *R. heilongjiangensis*, and one as *R. slovaca*, among pathogenic strains for guinea pigs. *R. sibirica* subsp. *sibirica* was detected in various NATT foci from *Dermacentor nuttalli*, *D. marginatus*, *D. silvarum*, *D. reticulatus*, *Haemaphysalis concinna* or *Ixodes persulcatus* ticks (Fig. 1). Among *R. sibirica* subsp. *sibirica* strains, two genotypes were identified, including the *sibirica sensu stricto* (25 strains) and BJ (two strains) genotypes. BJ-type strains had been isolated in the Russian Far East from *D. silvarum* ticks in 1981 and 1984, respectively. Neither strain differed in virulence and immunologic properties from *sibirica sensu stricto* strains. *R. heilongjiangensis* strains were detected in Siberia (Altay) and the Far East (Primorije region) from *H. concinna* ticks. The first of these two strains had been isolated by M. Schaiman in 1966 from ticks collected in Altay, 16 years before the first Chinese isolate. The only *R. slovaca* strain had been isolated in the Ural region by the same investigator in 1969.

Another 10 isolates, grown from *I. persulcatus* ticks, were identified as '*R. tarasevichiae*', a rickettsia initially detected using molecular methods only as 'Candidatus *R. tarasevichiae*' (Fig. 2) [4]. Strains from this rickettsia were isolated in Vero cells in which they caused a cytopathic effect.

Nine strains were identified as *R. raoultii*. These were classified within three genotypes, i. e., RpA4 (five isolates), DnS14 (one isolate), and DnS28 (three isolates) [5]. Initially found in Astrakhan (RpA4) and Altay (DnS14 and DnS28), these genotypes were later also detected in *Dermacentor* ticks in several NATT foci and NATT-free territories in Russia and Kazakhstan [1]. Strains were avirulent for guinea pigs and multiplied poorly in the yolk sacs of chicken embryos. Sera of infected guinea pigs did not cross-react with *R. sibirica* antigen.

DISCUSSION

The 50 rickettsial strains analysed in this study were classified within five species. Combined

Corresponding author and reprint requests: Nikolay V. Rudakov, Omsk Research Institute of Natural Foci Infections, Omsk State Medical Academy, 644080, prospect Mira, 7 Omsk, Russia
E-mail: rickettsia@mail.ru

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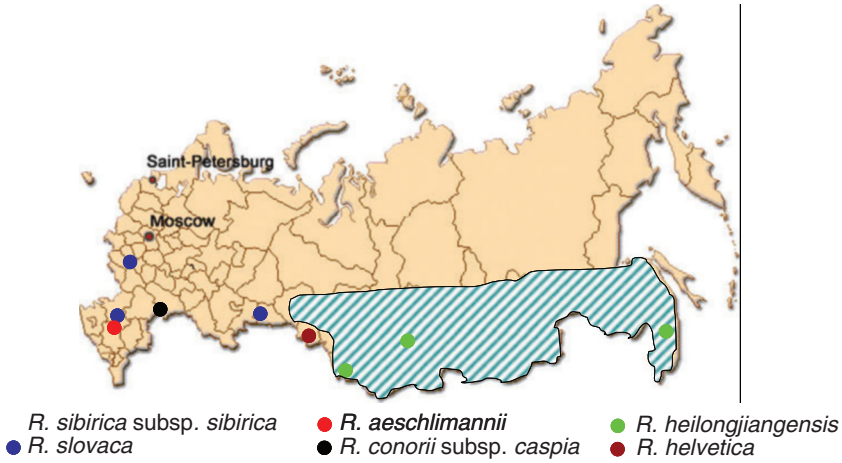

Fig. 1. Current knowledge on the geographic distribution of pathogenic rickettsial species in Russia.

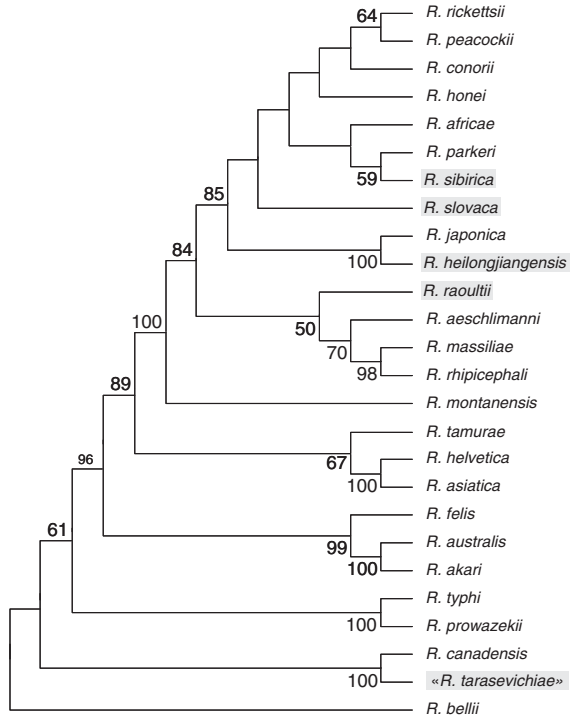

Fig. 2. Unrooted dendrogram showing the phylogenetic position of of rickettsial species identified in this study (shaded in grey), inferred from the comparison of *gltA* sequences by the neighbour-joining method. Bootstrap values greater than 50% are indicated at the nodes.

with previous data, six pathogenic species are currently known to be endemic in ticks in Russia (Fig. 1). Some of these species originated from NATT foci, which suggests that cases of rickettsiosis in these areas may be caused by several rickettsial species and not only *R. sibirica* subsp. *sibirica*. Future work will focus on another 47 as-yet uncharacterised rickettsial strains preserved in Omsk.

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